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STUDY OF POTENTIAL PROPHYLACTIC AND ANTIDOTAL USE OF SCAVENGING AGENTS IN TREATMENT OF CYANIDE POISONING

FINAL REPORT

Arthur S. Hume, Ph. D.

November 15, 1984
(January 1, 1983 - August 31, 1984)

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-83-C-3015

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2500 North State Street
Jackson, Mississippi 39216

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) STUDY OF POTENTIAL PROPHYLACTIC AND ANTIDOTAL USE OF SCAVENGING AGENTS IN TREATMENT OF CYANIDE POISONING		5. TYPE OF REPORT & PERIOD COVERED FINAL REPORT Jan. 1, 1983-Aug. 31, 1984 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Arthur S. Hume, Ph.D.		8. CONTRACT OR GRANT NUMBER(s) DAMD17-83-C-3015
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Pharmacology and Toxicology University of Mississippi Medical Center Jackson, Mississippi 39216		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62734A-3M162734A875-AJ.107
11. CONTROLLING OFFICE NAME AND ADDRESS U. S. Army Medical Research and Development Command Fort Detrick, Frederick, Maryland 21701-5012		12. REPORT DATE November 15, 1984 13. NUMBER OF PAGES 39
14. MONITORING AGENCY NAME & ADDRESS(If different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Distribution [redacted] to U.S. Government agencies and their contractors; administrative/operational use, July 8, 1985. Other requests for this document shall be referred to Commander, U.S. Army Medical Research and Development Command, ATTN: SGRD-RMS, Fort Detrick, Frederick, Maryland 21701-5012.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

cyanide, L-cysteine, N-acetyl-L-cysteine, toxicity, prevention, antidote keto acid, α-ketoglutaric acid, cyanohydrins, cytochrome oxidase, chemoprophylaxis.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The purpose of this project was to investigate the effectiveness of pretreatment with potential cyanide antagonists in protecting against the lethal effects of cyanide. The primary methodology used was LD_{50} studies in mice. Some studies involving intravenous infusion were done using rats.

Massive screening was avoided by selecting relatively nontoxic chemicals, most of which are capable of direct chemical reaction with cyanide. Of the thiol-like chemicals, cysteine and N-acetylcysteine were found to be effective, increasing the LD_{50} of cyanide, 127 and 158% respectively.

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However, the carbonyl compounds have proven to be a much more effective and promising group. Dehydroascorbic acid, pyruvic acid and α-ketoglutaric acid were observed to significantly protect mice against the lethality of cyanide. In vitro studies of the binding of these compounds to cyanide revealed correlation between protection against cyanide and the ability to bind cyanide.

Of the α-keto acids evaluated for their ability to protect animals against the lethal effects of cyanide, α-ketoglutaric acid was the most effective. The LD_{50} of cyanide was increased from 6 mg/Kg to 22 mg/Kg (a 400% increase).

These data indicate that α-ketoglutaric acid is the most potent single chemoprophylactic agent reported in the literature.

When a combination of α-ketoglutaric acid and sodium thiosulfate was administered to animals prior to injection of cyanide, the LD_{50} increased 20 fold compared to that in animals given saline pretreatment. This combination of α-ketoglutaric acid and sodium thiosulfate produced a 2.9-fold greater increase in LD_{50} than did the classic combination of sodium nitrite and sodium thiosulfate.

SUMMARY

The purpose of this project was to investigate the effectiveness of pretreatment with potential cyanide antagonists in protecting against the lethal effects of cyanide. The primary methodology used was LD_{50} studies in mice. Some studies involving intravenous infusion were done using rats.

Massive screening was avoided by selecting relatively nontoxic chemicals, most of which are capable of direct chemical reaction with cyanide. Of the thiol-like chemicals, cysteine and N-acetylcysteine were found to be effective, increasing the LD_{50} of cyanide, 127, and 158% respectively.

However, the carbonyl compounds have proven to be a much more effective and promising group. Dehydroascorbic acid, pyruvic acid and α-ketoglutaric acid were observed to significantly protect mice against the lethality of cyanide. In vitro studies of the binding of these compounds to cyanide revealed correlation between protection against cyanide and the ability to bind cyanide.

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FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. [NIH] 78-23, Revised 1978).

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I. Background and Statement of the Problem:

Antidoting cyanide intoxication is based upon the accepted mechanism of the action of cyanide. Cyanide binds to cytochrome oxidase, an enzyme component of the oxidative phosphorylation cascade in the mitochrondria. (1) This binding of cyanide to the heme iron results in inhibition of the activity of the enzyme and, thus, interrupts the utilization of oxygen and the transfer of electrons resulting in histotoxic hypoxia and death of the cell.

Presently, antidotal therapy used in the United States would include the use of a nitrite. (2) Nitrite converts the ferrous iron of hemoglobin to ferric iron (methemoglobin) for which cyanide has a higher affinity. (3) Thusly, methemoglobin can bind cyanide in the blood, preventing its access to cytochrome oxidase.

$$NO_2 + \text{hemoglogin} \longrightarrow \text{methemoglobin}$$

$$\text{methemoglobin} + CN \longleftrightarrow \text{cyanomethemoglobin}$$

The cyanide is released at a slow rate from the cyanomethemoglobin and is metabolized to thiocyanate

$$CN + S \xrightarrow{(E)} SCN \qquad E = \text{rhodanese}$$

Sodium thiosulfate is administered in cyanide intoxication to supply sulfur to the above reaction. The rate of metabolism is enhanced and the greatly less toxic thiocyanate is produced and excreted. (4)

An abundant source of sulfur atoms which can be reacted with cyanide is essential to the metabolism of cyanide to thiocyanate. (5)

Presently, thiosulfate is used for this purpose in treating cyanide poisoning. However, thiosulfate has disadvantages and a replacement is needed.

The combination of nitrite and thiosulfate as an antidote for cyanide intoxication is too slow in action, detoxifying capacity, ineffective for bound cyanide and, lowers blood pressure at a critical time.

The objective of these studies was to evaluate a non-methemoglobin - forming binding agent (α-ketoglutaric acid) of cyanide. This is desirable since the conversion of hemoglobin to methemoglobin reduces the oxygen carrying capacity of the blood and methemoglobinemia can result.

Several metal containing antidotes for cyanide are presently utilized in the treatment of cyanide poisonings. Hydroxocobalamin and cobalt edetate are most frequently used. Both agents are proposed to bind cyanide (6) (7). A methemoglobin former, dimethylaminophenol (DMAP) is also proposed as an antidote for cyanide poisoning (8).

Unfortunately, all these agents have disadvantages, solutions of hydroxocobalamin are very costly (9). Cobalt edetate can be toxic, i.e. produce cardiac arrhythymias and hypotension (9), dimethylaminophenol is rapidly active in producing methemoglobin but also releases cyanide too rapidly for effective use (10), cyanide is a potent nucleophile and may react

with carbonyl groups to form cyanohydrins (11). Thus, cyanide could react with the carbon in the ketone group of α-ketocarboxylic acids. Green and Williamson ascertained that pyruvic acid, an α-ketocarboxylic acid, reacts with cyanide in vitro to form cyanohydrin (12). Reduction in cyanide lethality has been observed after the administration of sodium pyruvate, which suggests cyanohydrin formation can protect against cyanide toxicity (13). Furthermore, Schwartz et al. have reported that sodium pyruvate enhances the efficacy of sodium nitrite and/or sodium thiosulfate in antagonizing the lethal effects of cyanide (14).

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II. Methods

Male ICR mice, weighing 23 to 28 g, were obtained from Charles River Laboratories, Inc. (North Wilmington, MA). Mice were given food and water ad libitum and were housed in a temperature-controlled environment with a photoperiod of 12 hours light/12 hours dark. Each experimental group was selected randomly from the general mouse population.

Potassium cyanide was obtained from the Aldrich Corporation (Milwaukee, WI). Sodium nitrite, pyruvic acid, ascorbic acid, α-ketoglutaric acid, cysteine, acetylcysteine, glutathione, homocysteine, disulfiram and hydroxycobalamin were obtained from Sigma Chemical Co., St. Louis, MO.

Fructose-1,6-diphosphate (FDP), ferroin, ammonium iron nitrate, ferric choline nitrate, ethyl propyl sulfide and 2,3-dimercapto-1-propanesulfonic acid were purchased from Pfaltz and Bauer Research Chem. Div. Pyridoxal HCl + dehydroascorbic acid was purchased from ICN, Plainville, NY. Sodium thiosulfate was purchased from Fisher Scientific Co., Springfield, NJ. All chemicals were reagent grade.

All solutions were prepared freshly in physiological saline (0.9% NaCl) on the day of experimentation. Solutions which were not to be used immediately were refrigerated and brought to room temperature before use. All solutions were kept in sealed containers. Antagonist chemicals were dissolved in saline, and the solution was adjusted to pH 7.4 with NaOH.

1. LD_{50} Studies

Pretreatment protocol was as follows: A dose of antagonist chemical was injected 10 minutes i.p. prior to the cyanide injection. Control animals were injected with saline for comparison in each experiment. Volumes of the injected solutions were kept small (0.1 ml/10 g of body weight) and the cyanide was always injected antipodal to the peritoneal region into which the cyanide antagonists were injected, in order to decrease any likelihood of intraperitoneal binding between cyanide and the cyanide antagonists. Cyanide was injected into mice (10 animals/dose) in varying doses in order to generate a lethality curve. LD_{50} values for cyanide were determined 12 hours after cyanide challenge. Dosages of the CN-antagonists were determined as the greatest doses which could be given without the appearance of toxic effects. These doses were determined prior to the actual experiments.

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2. Determination of Methemoglobin

Methemoglobin concentrations of blood samples were obtained from animals pretreated with antagonists as previously described. These animals were

killed at the time cyanide would have been administered. Blood samples collected from decapitated animals were analyzed for methemoglobin utilizing the method of Horecher-Brackett (1). Ten ml. of 0.32 M boric acid in 0.2 N sodium hydroxide was used to dissolve 100 mg of saponin which was subsequently diluted to 100 ml with distilled water. An aliquot volume of whole blood: borate-saponin buffer (1:10) was transferred to a cuvette and the optical density was read at a wavelength of 800 nm. A few crystals of potassium cyanide was subsequently added to the cuvette and the optical density was read again at 800 nm. The difference between the optical density values was utilized to calculate the concentration of methemoglobin in the blood sample. ANOVA was used to determine statistical significant between treatment groups.

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3. Determination of whole blood cyanide concentrations:

Blood cyanide levels were determined by gas chromatography utilizing the method of Darr, Capson, and Hileman (1980). One-tenth of a milliliter of concentrated phosphoric acid was placed into a one milliliter silanized reaction vial. The vial was subsequently capped, and two-tenths of a milliliter of heparinized blood was injected through the septum. The acid treated blood samples were vortexed for 30 seconds and then were placed into a 60° C water bath for 60 minutes. Five-hundred microliters of vial headspace were injected into a Hewlett-Packard series 5880A gas chromatograph equipped with a nitrogen phosphorous detector. The operating condition of the gas chromatograph was: inlet temperature, 250° C; carrier gas flow, 30 ml/min; air flow, 56 ml/min; helium-hydrogen flow, 27.5 ml/min. The 6-foot glass column will be packed with Porapak Q-S, 80-100 mesh.

Darr, H., Capson, T. and Hileman, F. Determination of hydrogen cyanide in blood using gas chromatography with alkali thermionic detection. Anal. Chem. 52: 1379-1381, 1980.

III. Studies of Prophylactic Antagonism of Lethal Effects of Cyanide.

Chemicals tested for their ability to prevent the lethal effects of cyanide (CN) were selected based principally upon their relative innocuousness and potential chemical reactivity with CN. Compounds containing reactive groups such as thiol, carbonyl and iron were evaluated in groups of mice. CN was injected in varying doses to establish a lethality curve. Potential antagonists were injected prior to CN challenge; LD_{50} data were collected and analyzed statistically.

In group A, of the sulfur compounds tested, cysteine, methionine, and N-acetylcysteine were selected because they are endogenous biochemicals. Glutathione administration was only slightly effective.

To probe the possibility of ferric-CN binding (as occurs with methemoglobin-CN and cytochrome oxidase-CN), four iron-containing compounds were evaluated. Three iron (ferric) salts were selected which are recognized as water soluble and non-irritating.

Group F compounds were designed to test the significance of the carbonyl group in the antagonism of CN. All of the compounds selected in this group occur naturally, pyridoxal HCl and ascorbic acids are vitamins. Pyruvic and α-ketoglutaric acids are endogenous and are involved in many status quo biochemical reactions in mammalian systems.

To test the feasibility of stimulating anaerobic metabolism in the cell to compensate for CN-induced hypoxia, fructose-1,6-diphosphate was selected. Hydroxocobalamin (Vitamin B_{12a}) binds cyanide to produce cyanocobalamin (Vitamin B_{12}) in a manner very similar to methemoglobin. This compound and sodium thiosulfate are evaluated as a known, presently-used antidote for cyanide. As scavenging agents, these compounds have distinct advantages therapeutically over classical antidotes, which require intermediate reactions to be effective. Sodium thiosulfate, which provides the sulfur substrate for thiosulfate sulfurtransferase, must first distribute to the portal circulation to interact with relevant enzymes. CN is neutralized by this sulfurtransferase only as it passes through the liver; hence, a delay of a few critical minutes occurs in the case of acute exposure (1). The other major antidote in current therapy is sodium nitrite, which acts by oxidation of hemoglobin to methemoglobin, which effectively binds CN. Sodium nitrite has disadvantages: It takes a relatively long time to elevate methemoglobin levels to a therapeutic level, and the compound is a vasodilator, so that a large dose could cause methemoglobinemia (2). Nitrite also, has a poor therapeutic ratio (effective dose/lethal dose) because the converted hemoglobin has no oxygen-carrying capacity. To summarize, present therapy for CN poisoning is invaluable but suffers from inherent risks and is subject to appreciable time delay. Use of scavenging agents in addition to, or in partial substitution for, elements of classical nitrite-thiosulfate therapy may save many lives in cases of acute exposure.

Agents Evaluated:

Following is a list of agents tested in a standardized manner in our laboratory for their effectiveness in reducing the toxicity of CN.

A. Sulfur containing Endogenous Compounds
   Cysteine
   Acetylcysteine
   Glutathione
   Homocysteine
   Methionine

B. Other Sulfur Compounds (not amino acids or congeners)
   Disulfiram
   Ethyl propyl sulfide

C. Alternate Biological Energy Source
   Fructose-1,6-diphosphate (FDP)

D. Iron Containing Compounds
   Ferroin
   Ammonium iron nitrate
   Ferric choline nitrate

E. Classical Antagonists for Efficacy Comparison
Sodium thiosulfate
Hydroxocobalamin

F. Carbonyl Compounds (possible cyanohydrin formers)
Pyruvic acid
Ascorbic acid
Dehydroascorbic acid
Pyridoxal HCl
α-Ketoglutaric acid

These agents include species from a number of different chemical groups which either have previously shown promise or might plausibly be effective as primary or adjunctive antidotes against CN poisoning (3), (4), (5). Major emphasis was placed on carbonyl and thiol compounds. Both of these groups contain closely related congeners which vary remarkably in their efficacy.

A. Evaluation of Chemoprophylaxis of Lethality of Cyanide by Sulfur Containing Endogenous Compounds.

In order to determine whether thiol- or sulfide-containing compounds (other than sodium thiosulfate) were active chemoprophylactic compounds against cyanide, certain compounds were selected for evaluation because of the chemical reactivity of the sulfur atoms contained in their structures, and their relatively low toxicity.

Since the lethality of cyanide varies substantially with age, size and associated changes in the physiology of test animals, an effort has been made to consistently use actively growing mice of 25-30 g (6).

1. Glutathione: Glutathione, a sulfur containing biochemical is involved in the metabolism of various xenobiotics. Since the metabolism of cyanide requires a supply of sulfur atoms, it was decided to determine whether the administration of glutathione prior to cyanide challenge would affect the lethality of cyanide. As shown in Table 1, glutathione (2 g/Kg i.p.) increased the LD_{50} of cyanide from 7.15 mg/Kg to 9.19 mg/Kg.

Table 1. Effects of Glutathione (2 g/Kg) on KCN Lethality in Mice[1]

Control				Prophylactic Treatment				
KCN (mg/Kg)	Dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	KCN (mg/Kg)	Dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	$\frac{LD_{50}\ compound}{LD_{50}\ control}$
6.5	2/12	7.15[2]	1.060	7.2	0/14	9.19[2]	1.094	1.285
7.2	8/12	(6.74-7.58)		8.0	1/6	(8.40-10.60)		
8.0	9/12			8.9	5/10			
8.9	6/6	n' = 36		9.9	4/6	n' = 28		
				11.0	5/6			
				12.2	6/6			

n' = the total number of mice per treatment group having survived percentages ranging from 16 - 84%.

[1] KCN was administered i.p. 15 minutes after i.p. dose of glutathione.

[2] 95% confidence interval.

2. Cysteine: Since cysteine had been reported in our first Annual Report (7) as effective but not significantly effective, other sulfhydryl-containing compounds very similar to cysteine in structure were evaluated. However, the data on cysteine are reported here for reference purposes.

Cysteine in doses of 150 mg/Kg i.p. increased the LD_{50} of cyanide from 7.26 to 9.29 mg/Kg, a 30% increase in LD_{50} (Table 2).

Table 2. Effects of Cysteine (150 mg/Kg) on KCN Lethality in Mice[1]

Control				Prophylactic Treatment				
KCN (mg/Kg)	Dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	KCN (mg/Kg)	Dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	$\frac{LD_{50}\ compound}{LD_{50}\ control}$
6.0	6/30	7.26[2]	1.04	7.5	2/30	9.29[2]	1.03	1.280
7.0	24/60	(6.97-7.55)		8.0	3/30	(8.99-9.61)		
7.5	37/60			8.5	0/30			
8.0	36/60	n' = 230		9.0	9/30	n' = 90		
8.5	16/20			9.5	21/30			
				10.0	21/30			

n' = the total number of mice per treatment group having survived percentages ranging from 16 - 84%

[1]KCN was administered 15 minutes after i.p. dose of cysteine.

[2]95% confidence interval.

When the dose of cysteine was administered (225 mg/Kg i.p.), the LD_{50} increased 45%, with a potency ratio of 1.453 when compared to saline.

Table 3. Effects of Cysteine (225 mg/Kg) on KCN Lethality in Mice[1]

Control				Prophylactic Treatment				
KCN (mg/Kg)	Dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	KCN (mg/Kg)	Dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	$\frac{LD_{50}\ compound}{LD_{50}\ control}$
6.5	3/10	7.30	1.14	7.5	0/10	10.61*	1.07	1.453
7.0	5/10	(6.41-8.32)		9.0	1/10	(9.90-1138)		
7.5	5/10			10.0	2/10			
8.0	10/10	n' = 30		11.5	8/10	n' = 20		

n' = the total number of mice per treatment group having survived percentages ranging from 16 - 84 %

[1]KCN was administered 15 minutes after i.p. dose of cysteine.

[2]95% confidence limits.

Neither of the doses of cysteine protected the animals against the lethality of cyanide sufficiently to warrant further study.

3. N-Acetylcysteine: Some work on N-acetylcysteine was reported in the first Annual Report (8). For comparison, we repeated our earlier studies; however, the dose of N-acetylcysteine was increased to 1 g/Kg body weight.

From Table 4, it is observed that N-acetylcysteine protected mice from the lethal effects of cyanide. The LD_{50} of cyanide increased from 7.30 to 11.53 mg/Kg, a 60% increase in LD_{50}.

Table 4. Effects of N-Acetylcysteine (1 g/Kg) on KCN Lethality in Mice[1]

Control				Prophylactic Treatment				
KCN (mg/Kg)	Dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	KCN (mg/Kg)	Dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	$\frac{LD_{50}\ compound}{LD_{50}\ control}$
6.5	3/10	7.30	1.14	7.5	0/10	11.53*	1.107	1.574
7.0	5/10	(6.41-8.32)		9.0	1/10	(10.42-12.76)		
7.5	5/10			10.0	2/10			
8.0	10/10	n' = 30		11.5	4/10	n' = 24		
				12.6	3/4			

n' = the total number of mice pertreatment group having survived percentages ranging from 16 - 84 %

[1]KCN doses were administered 15 minutes after the i.p. dose of N-acetylcysteine.

[2]95% confidence limits.

It is possible that the sulfhydryl compounds evaluated are serving as replacement sulfur donors in the metabolism of cyanide to thiocyanate.

4. Homocysteine: This homolog of cysteine increased the LD_{50} of cyanide from 7.15 to 8.94 mg/Kg. This chemical is not as effective as cysteine and much less effective than N-acetylcysteine. These results are reported in Table 5.

Table 5. Effects of Homocysteine (500 mg/Kg) on the KCN Lethality in Mice[1]

Control				Prophylactic Treatment				
KCN (mg/Kg)	dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	KCN (mg/Kg)	dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	$\frac{LD_{50}\ compound}{LD_{50}\ control}$
6.5	2/12	7.15[2]	1.060	8.0	0/10	8.94*[2]	1.15	1.250
7.2	8/12	(6.74-7.58)		8.9	3/6	(7.76-10.30)		
8.0	9/12			9.9	4/6			
8.9	6/6	n' = 36		11.0	5/6	n' = 18		

n' = the total number of mice per treatment group having survived percentages ranging from 16 - 84%

[1]KCN was administered i.p. 15 minutes after the i.p. injection of homocysteine.

[2]95% Confidence limits.

5. Methionine: This sulfur containing amino acid was evaluated as reported in Table 6; the administration of methionine increased the LD_{50} of cyanide from 7.15 mg/Kg to 10.40 mg/Kg (or 46%). The potency ratio when compared to saline was 1.455.

Although the increase in survivability of animals was significant, no further work on methionine was indicated because the efficacy of methionine was not considered to be an adequate improvement over other compounds of this group. It was proposed to synthesize derivatives of methionine in order to increase activity. (See MRDC Contract proposal)

Table 6. Effects of Methionine (500 mg/Kg) on the KCN Lethality in Mice[1]

Control				Prophylactic Treatment				
KCN (mg/Kg)	dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	KCN (mg/Kg)	dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	$\frac{LD_{50}\ compound}{LD_{50}\ control}$
6.5	2/12	7.15	1.060	8.9	1/6	10.40*	1.095	1.455
7.2	8/12	(6.74-7.58)		9.9	2/6	(9.50-11.39)		
8.0	9/12			11.0	4/6			
8.9	6/6	n' = 36		12.2	5/6	n' = 24		

n' = the total number of mice per treatment group having survived percentages ranging from 16 - 84%.

[1] KCN was administered i.p. 15 minutes after the i.p. injection of methionine.

[2] 95% Confidence limits.

B. Evaluation of Chemoprophylasis of Lethality of Cyanide by Other Sulfur Containing Compounds: Since sulfur atoms (sulfanes) are necessary to metabolize cyanide, an adequate supply of sulfur atoms must be maintained. Certain compounds were selected for the chemical environment of sulfur atoms within their structures.

If the electronic or chemical environment can result in a sulfur atom being more "labile," or reactive, it is possible to increase the rates of metabolism and excretion of cyanide.

1. Disulfiram and ethylpropyl sulfide

Disulfiram and ethylpropyl sulfide were studied. It is shown in Tables 7 and 8 that only minimal or no increase in LD_{50} of cyanide was effected. 2,3-dimercapto-1-propanesulfonic acid was also shown to afford no protection since six of seven animals died when 7.0 mg/Kg KCN was administered i.p. 15 minutes after a 1 g/Kg i.p. dose of this compound. (Six of ten animals died when saline was administered prior to KCN.)

Table 7. Effects of Disulfiram (300 mg/Kg) on KCN Lethality in Mice[1]

Control				Prophylactic Treatment				
KCN (mg/Kg)	Dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	KCN (mg/Kg)	Dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	$\frac{LD_{50}\ compound}{LD_{50}\ control}$
6.5	3/10	7.09	1.079	5.8	1/9	6.90	1.111	0.971
7.2	5/10	(6.57-7.65)		6.5	7/10	(6.20-7.67)		
8.0	8/10			7.2	2/10			
		n' = 30		8.0	9/10	n' = 20		

n' = the total number of mice per treatment group having survived percentages ranging from 16 - 84%.

[1]KCN was administered i.p. 15 minutes after i.p. dose of disulfiram.

[2]95% Confidence limits.

Table 8. Effects of Ethylpropyl Sulfide (830 mg/Kg) on KCN Lethality in Mice[1]

Control				Prophylactic Treatment				
KCN (mg/Kg)	dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	KCN (mg/Kg)	dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	$\frac{LD_{50}\ compound}{LD_{50}\ control}$
6.5	3/10	7.09	1.079	7.2	4/10	7.54	1.091	1.064
7.2	5/10	(6.57-7.65)		8.0	6/10	(6.91-8.22)		
8.0	8/10			8.9	9/10			
		n' = 30				n' = 20		

n' = the total number of mice per treatment group having survived percentage ranging from 16 - 84%.

[1]KCN was administered i.p. 15 minutes after i.p. dose of ethylpropyl sulfide.

[2]95% Confidence limits.

C. Evaluation of Chemoprophylaxis of Lethality of Cyanide by Alternate Biological Energy Source

1. Fructose-1,6-diphosphate.

Since cyanide produces a cytotoxic hypoxia, it was hypothesized that fructose-1,6-diphosphate, a substance recognized to protect tissue in hypoxic conditions, could be of benefit in cyanide-poisoned cells (9). The premise also suggests that fructose-1,6-diphosphate could assist in shifting the cell from oxidative to glycolytic metabolism, thus affording protection to the cell.

However, the results of our work (Table 9) indicate that fructose-1,6-diphosphate did not alter the lethality of cyanide. There is still the possibility that fructose-1,6-diphosphate could perform as an adjunct to other chemoprophylactic or antidotal agents.

Table 9. Effects of Fructose-1,6-diphosphate (1 g/Kg) on KCN Lethality in Mice[1]

Control				Prophylactic Treatment				
KCN (mg/Kg)	dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	KCN (mg/Kg)	dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	$\frac{LD_{50}\ compound}{LD_{50}\ control}$
6.5	1/10	7.05	1.081	6.5	0/10	7.16	1.182	1.016
6.75	4/10	(6.52-7.62)		6.75	3/10	(6.05-8.46)		
7.0	6/10			7.0	7/10			
7.3	4/10	n' = 40		7.3	5/10	n' = 50		
8.0	8/10			8.0	5/10			
				8.5	7/10			
				9.0	9/10			

n' = the total number of mice per treatment group having survived percentage ranging from 16 - 84%.

[1]KCN was administered i.p. 15 minutes after i.p. dose of fructose-1, 6-diphosphate.

[2]95% Confidence limits.

D. Evaluation of Chemoprophylaxis of Lethality of Cyanide by Ferric Iron Containing Compounds.

These experiments were based upon the premise that cyanide reacts with the ferric iron of cytochrome oxidase and methemoglobin. Therefore, ferric iron containing compounds could function as scavenging agents in the blood. Ferric-iron containing compounds which are soluble in water, relatively low in tissue irritation and relatively non-toxic were selected for evaluation. Ferric choline citrate, ammonium iron citrate and ferroin were tested in the routine LD_{50} screening procedure. Results of the tests are shown in Tables 10, 11, 12.

1. Ferric Choline Citrate, Ammonium Iron Citrte, and Ferroin

Ferric choline citrate (50 mg/Kg) was shown to afford no protection since six of six animals died when it was administered prior to an 8 mg/Kg dose of KCN.) However, some protection was afforded when the dose was increased to 150 mg/Kg (Table 10). Neither ammonium iron citrate or ferroin prevented KCN lethality at the doses administered.

Table 10. Effects of Ferric Choline Citrate (100 mg/Kg) on KCN Lethality in Mice[1].

Control				Prophylactic Treatment				
KCN (mg/Kg)	dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	KCN (mg/Kg)	dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	$\frac{LD_{50}\ compound}{LD_{50}\ control}$
5.47	1/10	6.15	1.103	6.75	4/6	7.05	1.239	1.147
6.08	4/10	(5.58-6.78)		7.50	2/6	(5.70-8.74)		
6.75	8/10			8.33	3/6			
7.50	8/10	n' = 30		9.26	1/6	n' = 30		
				10.30	1/6			

n' = the total number of mice per treatment group having survived percentages ranging from 16 - 84%.

[1]KCN was administered i.p. 15 minutes after ferric choline citrate, i.p.

[2]95% Confidence limits.

Table 11. Effects of Ammonium Iron Citrate (50 mg/Kg) on KCN Lethality in Mice[1].

Control				Prophylactic Treatment				
KCN (mg/Kg)	dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	KCN (mg/Kg)	dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	$\frac{LD_{50}\ compound}{LD_{50}\ control}$
5.47	1/10	6.15	1.103	6.75	3/6	6.74	1.092	1.096
6.08	4/10	(5.58-6.78)		7.50	5/6	(6.19-7.36)		
6.75	8/10							
7.50	8/10	n' = 30				n' = 12		

n' = the total number of mice per treatment group having survived percentage ranging from 16 - 84%.

[1]KCN was administered i.p. 15 minutes after ammonium iron citrate, i.p.

[2]95% Confidence limits.

Table 12. Effects of Ferroin (0.5 mg/Kg) on Lethal Effects of KCN[1].

Control				Prophylactic Treatment				
KCN (mg/Kg)	dead/ total	LD_{50}[2]	ED95/ED50	KCN (mg/Kg)	dead/ total	LD_{50}[2]	ED95/ED50	LD_{50} compound / LD_{50} control
5.47	1/10	6.15	1.103	6.75	4/6	6.22	1.166	1.011
6.08	4/10	(5.58-6.78)		7.50	5/6	(5.33-7.25)		
6.75	8/10							
7.50	8/10	n' = 30				n' = 12		

n' = the total number of mice per treatment group having survived percentages ranging from 16 - 84%.

[1]KCN was administered i.p. 15 minutes after ferroin, i.p.

[2]95% Confidence limits.

E. Evaluation of Chemoprophylaxis of Lethality of Cyanide by Sodium Thiosulfate and Hydroxocobalamin.

1. Sodium Thiosulfate

In order to compare the efficacy of the test compounds with that of a well-established chemoprophylactic agent, sodium thiosulfate was evaluated. The administration of sodium thiosulfate to mice prior to cyanide challenge increased the LD_{50} of cyanide from 7.05 mg/Kg to 17.62 mg/Kg, an increase of 250%. The potency ratio (compared to saline) was 1.50. As was expected, a significant protection against the lethal effects of cyanide was afforded by sodium thiosulfate. The results of these experiments are shown in Table 13.

Table 13. Effects of Sodium Thiosulfate (1 g/Kg) on KCN Lethality in Mice[1].

Control				Prophylactic Treatment				
KCN (mg/Kg)	dead/ total	LD_{50}[2]	ED_{95}/ED_{50}	KCN (mg/Kg)	dead/ total	LD_{50}[2]	ED_{95}/ED_{50}	LD_{50} compound / LD_{50} control
6.5	1/10	7.05	1.081	15	2/10	17.62*	1.110	2.50
6.75	4/10	(6.52-7.62)		18	6/10	(15.87-19.56)		
7.0	6/10			20	7/10			
7.3	4/10	n' = 40				n' = 30		
8.0	8/10							

n' = the total number of mice per treatment group having survived percentage ranging from 16 - 84%.

[1]KCN was administered i.p. 15 minutes after sodium thiosulfate, i.p.

[2]95% confidence limits.

2. Proposed Prophylactic Agents in Combination with Nitrite/Thiosulfate Therapy

Rats were infused via tail vein with either saline, fructose-1, 6-diphosphate, N-acetyl-L-cysteine, ascorbic acid or sodium thiosulfate at the rate of 0.5 ml/min in a volume of 1 ml per 300 g body weight to yield final dosages as indicated in Table 12. Immediately following this infusion, rats were administered the following in rapid succession:

KCN (12 mg/kg, ip)
Sodium nitrite (100 mg/kg, ip)
Sodium thiosulfate (1,000 mg/kg, iv, same rate and volume as pretreatment, above).

When injection of sodium nitrite and infusion of sodium thiosulfate immediately followed KCN treatment, about 85% of the rats died. Because LD_{50} curves of KCN tend to be sharp, a dosing regime in this range should have higher survival rates when only a small added degree of protection was afforded by prophylactic agents. It is apparent that none of the agents tested improved survival over that afforded by nitrite/thiosulfate treatment alone.

Table 14. Prophylactic Effect of Various Agents to Doses of 12 mg/kg KCN as Adjuncts to Thiosulfate/Nitrite Therapy in Rats[1*]

Pretreatment	mg/kg i.p.			
	KCN	$NaNO_2$	$Na_2S_2O_3$	Mortality
Saline	12	100	1,000	13/15
Fructose-1,6-diphosphate (150 mg/kg)	12	100	1,000	8/9
N-Acetyl-L-cysteine (1 g/kg)	12	100	1,000	6/6
Ascorbic acid (150 mg/kg)	12	100	1,000	7/8
Sodium thiosulfate (1 g/kg)	12	100	1,000	5/5

[1*] Treatments progressed from left to right as follows:
Pretreatment was i.v. infusion at a rate of 0.5 ml/min, total volume given averaged 0.6 ml. KCN was administered ip immediately following pretreatment. Sodium nitrite was i.p. immediately following KCN. Sodium thiosulfate was given i.v. immediately following KCN, at the same rate and to the same final volume as pretreatment.

3. Hydroxocobalamin

Hydroxocobalamin, administered in the dosage used in these studies resulted in an increase of LD_{50} values of KCN i.p. from 8.95 mg/kg to 17.22 mg/kg. This represents a 2 fold increase in LD_{50} value.

Table 15. Effects of Hydroxocobalamin HCl (250 mg/Kg) on KCN Lethality in Mice[1]

LD_{50} of KCN (mg/kg)	
Control	
8.95 (8.65 - 9.26)[2]	17.22 (14.25 - 20.81)[2]
n' = 18	n' = 14

n' = the total number of mice per treatment group having survived percentages ranging from 16 - 84%.

[1]Hydroxocobalamin administered i.v. immediately before i.p. administration of KCN.

[2]95% Confidence limits.

F. Studies on Chemoprophylaxis of Antagonism of Lethality of Cyanide by Carbonyl Compounds.

With the finding that some carbonyl-containing compounds are effective in preventing the lethal effects of cyanide, it was deemed desirable to study other carbonyl compounds. α-Keto acids, carbonyl-containing compounds, appeared to be likely candidates, as pyruvic acid and ascorbic acid had been reported effective in antagonizing the lethal effects of CN. (10) (11)

1. LD_{50} Studies:

Table 16 indicates LD_{50} values for mice pretreated with antagonists 15 minutes prior to KCN challenge and also includes the LD_{50} values for saline-pretreated controls tested concurrently. The 95% confidence intervals for both groups are presented. Dehydroascorbic acid, pyruvic acid and α-ketoglutaric acid increased the LD_{50} value of KCN. No protection against the effects of CN was afforded by preinjections with 150 mg/Kg bromopyruvic acid, 300 mg/Kg pyridoxal HCl, or 500 mg/Kg ascorbic acid. Significance ($p < 0.05$) is indicated by an asterisk.

Groups of mice were injected with varying doses of CN to establish a lethality curve. (See Methods) α-ketoglutaric acid (1 g/Kg) was injected i.p. 15 minutes prior to CN challenge. The number of dead animals per group were determined for 24 hours and recorded and statistically analyzed.

Table 16. Effects of Carbonyl Compounds on KCN Lethality in Mice

	LD_{50} VALUES (95% CONFIDENCE INTERVAL) (MG/KG, I.P.)
SALINE	7.26 (6.97-7.55)
ASCORBIC ACID	NO PROTECTION
SALINE	7.15 (6.74-7.58_
DEHYDROASCORBIC ACID	13.08 (11.64-14.94)*
SALINE	7.11 (6.74-7.50)
PYRIDOXAL HCL	NO PROTECTION
SALINE	7.15 (6.74-7.50)
PYRUVIC ACID	11.36 (10.43-12.29)*
SALINE	8.0 (7.46-8.55)
α-KETOGLUTARIC ACID	39.0 (35.10-43.20)*

2. Determinations of cyanide in buffered aqueous solutions following addition of carbonyl compounds:

Each of the six carbonyl compounds was dissolved in 0.4 M sodium phosphate buffer, pH 7.0, to yield a carbonyl compound concentration of 40 mM. KCN was dissolved in water to yield a final concentration of 20 mM. At time zero, 1 ml

KCN solution was added to 1 ml of carbonyl compound solution and periodic samples were withdrawn and injected into a liquid chromatograph (Model M6000A, Water, Milford, MA) equipped with an Au/Hg electrochemical detector (Bioanalytical Systems, Lafayette, In). A 10-μm C-18 reversed-phase column was used with 0.1 M monochloroacetic acid as the mobile phase. For assays involving ascorbic acid, 1 mM octylamine was added to the mobile phase to separate ascorbic acid and CN peaks. The Au/Hg electrode was set at +0.15 volt relative to the Ag/AgCl reference electrode. The number and frequency of measurements were dictated in some cases by the presence of interfering peaks; however, measurements were made over a 15 to 30 minute time period.

3. Disappearance of cyanide from buffered solution containing carbonyl compounds:

Table 17 indicates a range of reactivity of antagonists with CN. Pyruvic acid and dehydroascorbic acid are clearly very reactive with KCN, in terms of both initial reaction rate and final residual KCN at or near equilibria. α-Ketoglutaric acid is apparently less reactive than pyruvic acid and dehydroascorbic acid. Of the compounds tested, ascorbic acid showed little promise as an effective antagonist.

Table 17. Disappearance of Cyanide in pH 7.0 Buffer Containing Initial Concentrations of 10 nM KCN and 20 mM of Potential Antagonists

	α-KA	PA	PYX	AA	DAA
Time to disappearance of 50% of initial cyanide (min)	4.4	1.0	5.1	--	1.0
Minimum cyanide levels (% of initial)	8.0	6.0	22	91	10
At time (min)	(13)	(16)	(14)	(19)	(12)

α-KA α-KETOGLUTARIC ACID PYX PYRIDOXAL. HCL

PA PYRUVIC ACID AA ASCORBIC ACID

DAA DEHYDROASCORBIC ACID

4. Determination of the disappearance of cyanide from blood containing carbonyl compounds:

KCN was added to 0.50 ml portions of mouse blood to obtain a final concentration of 450 nmol/ml (See figure legend at bottom of page -450 μmol/ml = 450 μN. This level is equivalent to the blood concentration expected following an i.p. injection of approximately 25 mg/Kg of KCN. Dose is based upon the data derived from another study (12). Carbonyl compounds were added to the vials to obtain a final concentration of 2 mg/ml of compound. Samples were capped and incubated as previously described. (See Methods) Control samples contained the same amount of CN as test samples, but did not contain carbonyl compounds. Data were evaluated by one-way ANOVA with $p < 0.05$ as the criterion for significance.

Figure 1 indicates the percentage of CN released into the headspace of blood samples following a 1.5 hour incubation at 30°C. Initial levels of KCN and carbonyl compounds had been selected to approximate blood levels following treatment for a serious poisoning incident.

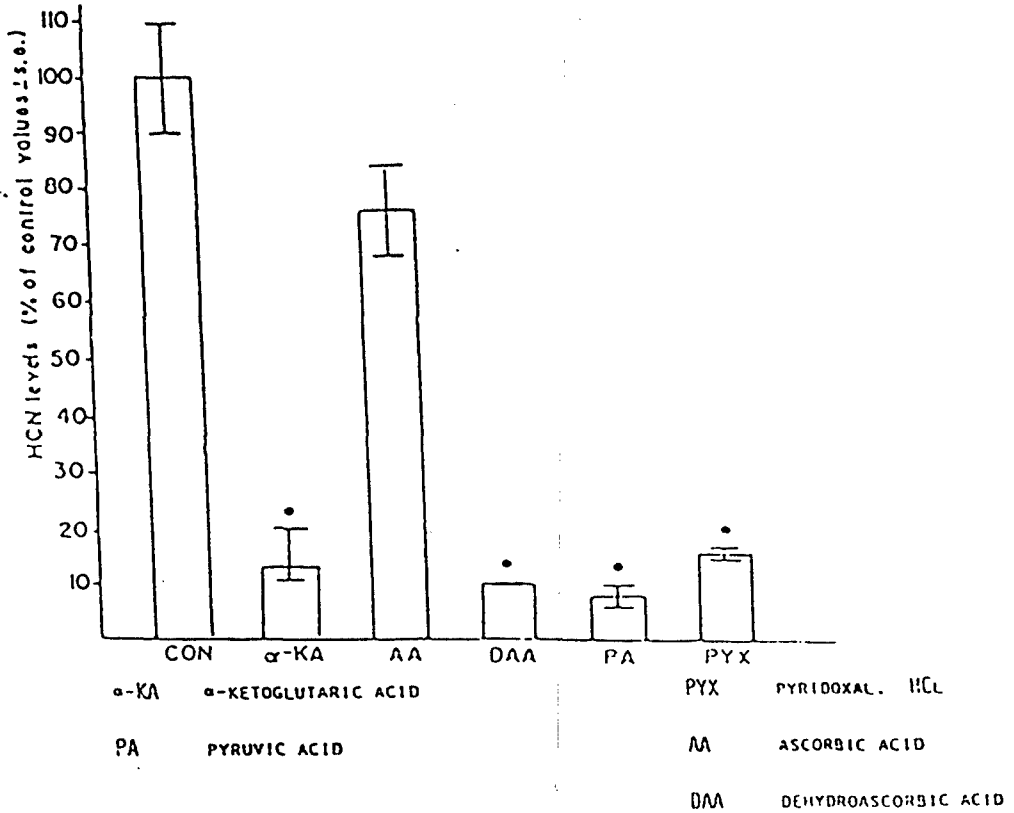


Figure 1. Percentage of cyanide in blood samples, to which 0.45 μM KCN and 2 mg/ml of respective antagonists were added. Samples drawn following 1.5 hours incubation at 37° C.

5. Efficacy of Chemoprophylaxis Against the Lethal Effects of Cyanide by α-Ketoglutaric Acid:

In this study, α-ketoglutaric acid, an α-ketocarboxylic acid, was evaluated for its ability to counteract the lethal effects of CN. Pretreatment with α-ketoglutaric acid was observed to increase the LD_{50} value of CN (32 mg/Kg) by a factor of 5, a value not significantly different from that ascertained in mice pretreated with sodium thiosulfate and sodium nitrite. It is concluded from these studies that α-ketoglutaric acid provides a greater degree of protection than sodium nitrite and sodium thiosulfate without the dangerous formation of methemoglobin.

Table 18. Effects of α-Ketoglutaric Acid (2 g/Kg) on the KCN Lethality in Mice[1]

Control				Prophylactic Treatment				
KCN (mg/Kg)	dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	KCN (mg/Kg)	dead/ total	LD_{50}[2]	$\frac{ED_{95}}{ED_{50}}$	$\frac{LD_{50}\ compound}{LD_{50}\ control}$
7.0	2/10	8.04	1.19	10.0	0/10	34.83	1.79	4.33
7.5	3/10	(7.53-8.59)		2.0	0/10	(29.05-41.74)		
8.5	6/10			3.8	4/10			
9.0	8/10	n' = 40		4.5	9/10	n' = 30		

n' = the total number of mice per treatment group having survived percentages ranging from 16 - 84%

[1]KCN was administered i.p. 15 minutes after the i.p. injection of homocysteine.

[2]95% Confidence limits.

6. Efficacy of Chemoprophylaxis of Lethal Effects of Cyanide by α-Ketoglutaric Acid Compared with Sodium Nitrite and/or Sodium Thiosulfate.

Table 19 presents a comparison of the potency ratios among the components of four regimens differing only in the addition of α-ketoglutaric acid to the protocol. This potency ratio is defined as the LD_{50} value of CN including α-ketoglutaric acid in the pretreatment regimen divided by the LD_{50} value of CN without α-ketoglutaric acid in the pretreatment regimen. Pretreatment with α-ketoglutaric acid increased the LD_{50} value of CN in mice almost 5-fold. The addition of α-ketoglutaric acid to the sodium nitrite pretreatment approximately doubled the potency ratio. The potency ratio of sodium thiosulfate plus α-ketoglutaric acid pretreatment was approximately 6 times greater than that for sodium thiosulfate alone. A 3.5-fold increase in protective ratio was shown for pretreatment with α-ketoglutaric acid following sodium nitrite, and sodium thiosulfate as compared to pretreatment with only sodium nitrite and sodium thiosulfate.

TABLE 19. Comparison of the Effects of α-Ketoglutaric Acid on Pretreatment Regimen for Cyanide Intoxication.

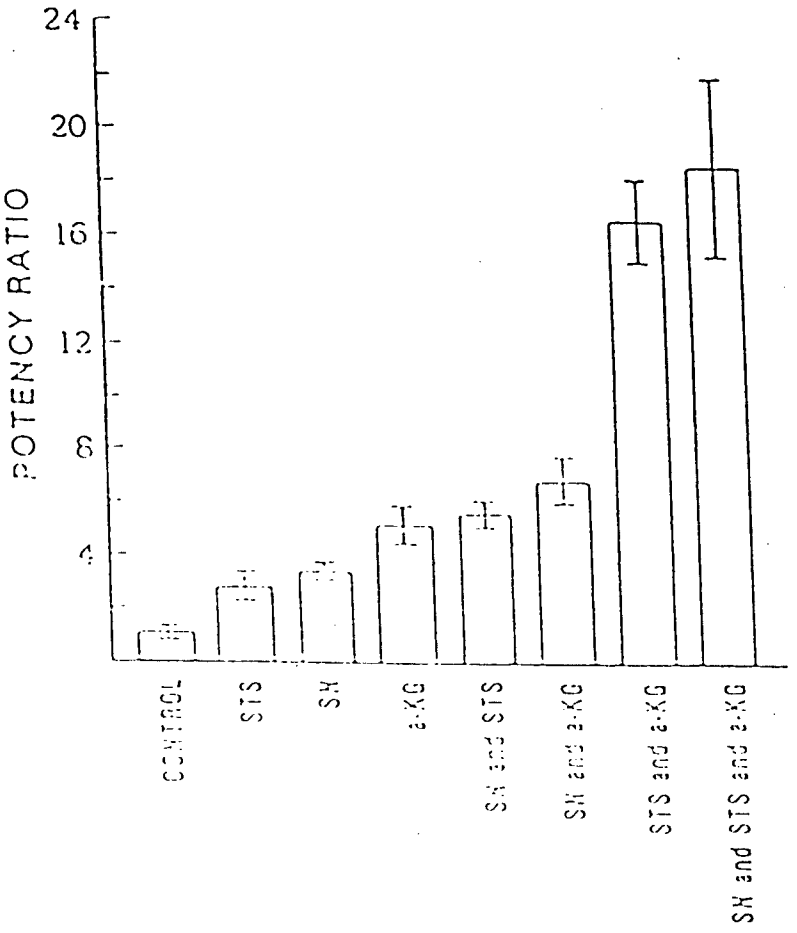
Group	Pretreatment Regimen	Potency Ratio[a]
A.	Controls	4.96 (4.50-5.45)
B.	α-ketoglutaric acid	
A.	$NaNO_2$	2.04 (1.85-2.24)
B.	$NaNO_2$ + α-ketoglutaric acid	
A.	$Na_2S_2O_3$	6.17 (5.37-7.10)
B.	$Na_2S_2O_3$ + α-ketoglutaric acid	
A.	$NaNO_2$ + $Na_2S_2O_3$	3.50 (3.18-3.85)
B.	$NaNO_2$ + $Na_2S_2O_3$ + α-ketoglutaric acid	

[a]Potency ratio = $\frac{LD_{50}\ Value\ of\ KCN\ in\ Group\ B}{LD_{50}\ Value\ of\ KCN\ in\ Group\ A}$

Doses used: α-ketoglutaric acid 2 g/Kg i.p.
Sodium Nitrite ($NaNO_2$) 100 mg/Kg s.c.
Sodium thiosulfate ($Na_2S_2O_3$) 1 g/Kg i.p.

Figure 2 shows the potency ratios of each protocol. The potency ratio is defined as the LD_{50} value of CN in the particular pretreatment regimen divided by the LD_{50} value of CN in control saline-treated animals. It is apparent from this figure that α-ketoglutaric acid pretreatment protected as well as or better than any pretreatment regimen that lacked α-ketoglutaric acid. The addition of sodium nitrite to the α-ketoglutaric acid pretreatment resulted in increased protection, albeit in less than additive terms. The addition of sodium thiosulfate treatment to either the α-ketoglutaric acid or the sodium nitrite plus α-ketoglutaric acid pretreatment regimen resulted in more than additive protection. The addition of sodium nitrite to the sodium thiosulfate plus α-ketoglutaric acid pretreatment did not produce a statistically significant elevation in the potency ratio.

FIGURE 2.



STS = Sodium Thiosulfate
SN = Sodium Nitrite
aKG = α-Ketoglutaric acid

Figure 2. Potency Ratios of Combinations of Antidotes of KCN Lethality in Mice.

Potency Ratio = LD_{50} Value of KCN with Antidotal Pretreatment/LD_{50} Value of KCN

7. Possible Production of Methemoglobin by α-Ketoglutaric Acid

Table 20 demonstrates that the protection by α-ketoglutaric acid against CN intoxication, unlike those of sodium nitrite, is not due to methemoglobin formation. No statistically significant difference in whole blood methemoglobin levels (g/100ml) was observed between control mice and those mice pretreated with α-ketoglutaric acid. No difference in methemoglobin levels was found between control animals and sodium thiosulfate-pretreated animals. α-ketoglutaric acid given in addition to sodium nitrate did not increase the amount of methemoglobin formed after administration of sodium nitrite alone. Moreover, α-Ketoglutaric acid did not augment methemoglobin levels when added to the sodium nitrite plus sodium thiosulfate pretreatment regimen.
(See Methods section for procedures used to determine methemoglobin levels.)

Table 20. Effects of Pretreatment Regimen on the Production of Methemoglobin

Pretreatment Regimen	Grams % Methemoglobin
Controls	$0.22 \pm .16$
α-ketoglutaric acid	$0.28 \pm .10^{a}$
$NaNO_2$	$3.43 \pm .32^{*}$
$NaNO_2$ + α-ketoglutaric acid	$2.48 \pm .22^{*b}$
$Na_2S_2O_3$	$0.10 \pm .10$
$Na_2S_2O_3$ + α-ketoglutaric acid	$0.19 \pm .10^{c}$
$NaNO_2$ + $Na_2S_2O_3$	$3.68 \pm .40^{*}$
$NaNO_2$ + $Na_2S_2O_3$ + α-ketoglutaric acid	$3.35 \pm .69^{*d}$

*$p < 0.05$ compared to values of control animals.
[a]$p > 0.05$ compared to values of control animals.
[b]$p > 0.05$ compared to values of $NaNO_2$-pretreated animals.
[c]$p > 0.05$ compared to values of $Na_2S_2O_3$-pretreated animals.
[d]$p > 0.05$ compared to values of $NaNO_2$ + $Na_2S_2O_3$-pretreated animals.

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IV. Results and Discussion

The rapid onset of toxic effects, including lethality by cyanide demand rapid action by the antagonistic agents. Therefore, it appeared that an agent which will bind cyanide in the blood and prevent its entry into the cells would be the most beneficial in cyanide poisoning. If enhancement of metabolism can be accomplished this could reduce the concentration of cyanide.

The sulfides and thiol compounds studied in this project were only weakly antagonistic to cyanide. It can be considered that sulfur only weakly binds cyanide, if at all. However, this work does not eliminate their potential use as sulfur donors in the metabolism of cyanide.

It is reported that cyanide has a greater affinity for the ferric form of iron (methemoglobin and cytochrome a) than for the ferrous iron (hemoglobin) (1). However, no ferrous or ferric-iron compound evaluated in this study reduced KCN lethality significantly. Apparently, CN is attracted to the ferric iron but the ferric-iron must be present in the heme structure for CN - Fe^{+++} interaction to take place.

Another well recognized reaction in organic chemistry was the basis for the study of keto acids. This reaction of a carbonyl group (C=O) and CN results in the formation of a cyanohydrin (2). This reaction occurs spontaneously with more keto acids. Also, Cittanini had reported that pyruvic acid would protect mice from KCN lethality (3). Further studies by Schwartz, et al. confirmed this effectiveness (4). So, keto acids of low toxicity were selected to evaluate ascorbic acid, and dehydroascorbic acid showed some promise, but ascorbic acid required rather large doses and dehydroascorbic acid is toxic in CN antagonizing doses. However, an endogenous alpha keto acid, α-ketoglutaric acid was significant in its protectiveness.

From Table it is observed that the LD_{50} values was increased from 8.04 mg/kg to 34.8 mg/kg.

Work in our laboratory had shown that α-ketoglutaric acid would bind cyanide completely. Studies into the possible mechanism of action of carbonyl compounds show that cyanide is found by carbonyl compounds. Cyanide was shown to be retained in buffered solutions and blood when a carbonyl compound is added. Binding of cyanide would be similar to that of methemoglobin in the treatment of cyanide poisoning.

In vivo studies showed that α-ketoglutaric acid would protect mice from the lethality approximately equal to sodium nitrite/sodium thiosulfate combination. It is shown that if sodium (thiofate is added to the α-ketoglutaric acid the LD_{50} is increased to 101.3 mg/kg. This is a 9 fold increase in the LD_{50} of the controls and a 3 fold increase in the LD_{50} of the presently used sodium nitrite) sodium thiosulfate combination, and a 3 fold increase in LD_{50} of alpha keto glutaric acid alone. From these data we can conclude that α-ketoglutaric acid is superior to sodium nitrite as a binding agent but the protective ability is enhanced by the addition of a sulfur donor, sodium thiosulfate.

Further studies should be underway to elucidate the mechanism of action of α-ketoglutaric acid in antagonizing cyanide. The pharmacokinetics of this compound should be studied to determine peak plasma levels and their time of occurrence. Also, the duration of action could be determined by these studies in order to determine a pattern of dosages which would provide a continous protective blood level.

Pre-exposure and post exposure studies in preventing and treating cyanide intoxication should be carried out. Data should be collected in higher animals as soon as possible, since studies involving humans will be accompolished only in an actual situation. Animal data must be convincing if clinicians are to use α-ketoglutaric acid in the emergency situation.

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